



Research Article

NUTRITIONAL AND PHYTOCHEMICAL CONTENT OF INDIGENOUS LEAFY VEGETABLES CONSUMED IN BOTSWANA

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ABSTRACT

Plant materials especially green leafy vegetables contain nutrients and phytochemicals whose consumption has been associated with protecting the human body from chronic diseases. With the aim to promote the utilisation of indigenous leafy vegetables commonly consumed in Botswana, nutrient and phytochemical composition of three leafy vegetables: Amaranth (*Amaranthus spp.*), Spider plant (*Cleome gynandra*), and Cowpea leaves (*Vigna unguiculata*) were evaluated. The moisture content of the vegetables was high ranging from 84.1 ± 0.05 (*Amaranthus spp.*) to $88.8 \pm 0.10\%$ wet basis (*Cleome gynandra*). The ash content ranged between $1.90 \pm 0.06\%$ (*Amaranthus spp.*) and $3.0 \pm 0.04\%$ (*Cleome gynandra*). All the leafy indigenous vegetables were found to be poor sources of protein, fat and fibre. The total phenolics ranged from 10.4 ± 0.5 to 40.4 ± 0.11 mg/g DW. *Amaranthus spp.* had the highest phenolic content (40.4 ± 0.11 mg/g DW). 3,4-dihydroxybenzoic, chlorogenic and ferulic acids were found to be present in *Amaranthus spp.*, 3,4-dihydroxybenzoic, 4-hydroxybenzoic, p-coumaric and ferulic acids were found in *Vigna unguiculata* whilst vanillic, chlorogenic and ferulic acids were found in *Cleome gynandra*. Ferulic acid appeared in all the samples analysed, however, chlorogenic acid was the most abundant. The results from the study emphasize the role of these vegetables as a source of nutrients and polyphenols which could contribute to their health promoting properties and offer enormous opportunities for the functional food industry.

KEY WORDS: Indigenous leafy vegetables, Phenolic compounds, HPLC, Proximate analysis, *Vigna unguiculata*, *Cleome gynandra*, *Amaranthus spp.*

INTRODUCTION

Diets rich in fruit and vegetables have been associated with physical wellbeing. In addition, epidemiological studies indicate that increased intake of leafy vegetables is associated with decreased risk of cancers, cardiovascular disease, cataract, macular degeneration and other age-related diseases [1]. Millions of people in many developing countries do not have enough food to meet their daily requirements and a further more people are deficient in one or more micronutrients [2]. In most cases rural communities depend on wild resources including wild edible

plants to meet their food needs in periods of food crisis. Integrating wild vegetables into diets has been promoted as one of the most food based strategies to achieve optimal dietary requirements to combat micronutrient deficiencies in a sustainable way [3]. Indigenous leafy vegetables (ILV's) have long been part of traditional diets in communities worldwide, yet many of these crops are underutilized and their nutritional value is unknown [4]. However, several studies have indicated that ILV's consumed in Africa contain higher level of micronutrients than those found in most exotic areas [5,6,7]. Dietary intake of ILV's have been reported to have healing properties, serve as a source of micronutrients [3,8] and reduce the risk of cardiovascular diseases and other degenerative diseases [9].

Apart from nutritive value, ILV's contain phenolic compounds that have been shown to have antioxidant properties [10]. A study done in sub-Saharan Africa found that *Cleome gynandra*, *Amaranthus spp.* and *Solanum macrocarpon* showed high antioxidant activity with 1.56, 1.0 and 0.87 mmol TE/100g, respectively [11]. The dominant phenolic compounds presents in ILV's are the flavonoids, phenolic acids, lignans, lignins, condensed tannins, coumarins and hydrolysable [10]. These compounds prevent many chronic diseases associated with cancer, inflammation, atherosclerosis, and aging caused by free radicals [7,12,13]. The antioxidant properties of plant foods have been attributed to their phenolic content, mostly flavonoids and phenolic acids which allow them to act as free radical scavengers and protect cells from oxidative damage induced by free radicals and thereby help to protect against oxidative stress [14,15]. They are known to reduce cholesterol production in the body thereby helping to keep the blood pressure down [16].

Although most of the compositional aspects of ILV's commonly consumed in sub-Saharan are known, available data on the phenolic composition and antioxidant activity of some of these ILV's is limited [7,11,12]. To the best of our knowledge, no HPLC method has been reported for the determination of phenolic acids in ILVs of Botswana. Information is however scanty on the nutritional and phytochemical contents of these leafy vegetables. Hence, there is need to discover the potential of our local vegetables in relation to the provision of basic nutrients and phytochemicals, as this will help provide vital data for food processors, nutritionist, dieticians, as well as the consumers. Furthermore, evaluating the nutritional importance of indigenous vegetables can lead to a better understanding of the value of these plants [17]. Aiming to promote the production and consumption of ILV's, the purpose of the study was to evaluate the proximate nutrient content, and the phenolic profile and phenolic content of extracts of *Amaranthus spp.*, *Cleome gynandra*, and *Vigna unguiculata* commonly consumed in Botswana..

MATERIALS AND METHODS

Plant materials

Three indigenous leafy vegetables namely Amaranth (*Amaranthus spp.*), Spider plant (*Cleome gynandra*), and Cowpea leaves (*Vigna unguiculata*) were collected in the North Eastern parts of Botswana [21°10'25"S & 27°30' 45"E]. The vegetables represent the best known and generally the most widely consumed indigenous leafy vegetables in Botswana especially in rural communities [13]. Often these vegetables are cooked and eaten as a relish together with a starchy staple food, usually in the form of porridge.

Sample Preparation

Five hundred grams (500 g) of the leaves were thoroughly washed with fresh running water to remove surface dirt and sun dried for proximate determination phytochemical analysis. The samples were ground into powder and stored at -40 °C until analysis.

2 Determination of Nutritional Composition

Moisture, ash, proteins, fat and dietary fiber were determined using Association of Official Analytical Chemicals (AOAC, 1990) [18].

Determination of % Moisture Content Front Matter

The moisture content was determined by using the oven-dry method. Briefly, 2 g of sample was accurately weighed into a moisture dish in triplicate. The sample was dried for 24 hrs. at 105 °C. After drying, the samples were removed from the oven and placed in a desiccator to cool to constant weight. The measurements were expressed as percent of dry weight in triplicate.

Determination of % Ash

Four grams of the leaf sample was weighed into a porcelain dish that had previously been weighed. This was dried at 105 °C and ignited at 500 °C until light grey ash was obtained. This was removed from the muffle furnace, placed in a desiccator until it cooled, and was then weighed.

Determination of Protein

Crude protein was estimated by the Kjeldahl method. Five grams of the sample was weighed into a 1 L Kjeldahl flask and digested with 10 g potassium sulphate, copper sulphate catalyst tablets and 25 mL concentrated sulphuric acid. The mixture was then heated for an additional 60 min, cooled and 400 mL distilled water added. After addition of 80 mL of 40% sodium hydroxide into the digested sample the 1 L Kjeldahl flask was heated in order to liberate ammonia. The ammonia was collected into a boric acid solution (40 mg/L) that contained an indicator made up of 0.5 g bromosocresol green and 0.1 mL methyl red in 100 mL of 95% ethanol. Heating continued until 50 mL distillate was collected. The boric acid mixture was titrated against 0.1 M HCl until a faint pink colour persisted. The blank contained reagents only and the titrate value was subtracted from the sample value.

Determination of % Crude Fat

Five grams (5 g) of the ground leaf sample was placed in a thimble lined with a circle of filter paper and then in a 50 ml beaker and dried in an oven for 6 hours at 105 °C. The thimble with its contents was transferred to a Soxhlet extractor and extracted with ethyl ether for 6 to 8 hours at a condensation rate of 3 - 6 drops per second. At the completion of the extraction, the fat extract was transferred from the extraction flask into a pre-weighed evaporating dish with several rinsing of ethyl ether. The evaporating dish was placed in a fume hood with the fan on, to evaporate the ethyl ether until no odour was detectable. The dish with its contents was dried in an oven for 30 minutes at 105 °C, removed from the oven, cooled in a desiccator to constant weight and weighed.

Determination of % Crude Fibre

Five grams (5 g) of ground sample was weighed and placed in a 1 litre conical flask and then 150 ml pre-heated 0.128 M H₂SO₄ was added and the content boiled for 30 minutes. The content was filtered through fluted funnel and the residue washed three times with hot water. One fifty milliliters of preheated 0.15 M KOH was then added and heated to boil. A few drops of antifoaming agent (n-octanol) was added and the mixture was boiled slowly for 30 minutes, filtered and the residue washed three times with hot water, followed by washing three times with acetone in Cold Extraction Unit (Tecator1615). The resulting residue was dried in the oven at 130 °C for 1 hr., cooled in a desiccators and weighed, and then ashed at 500 °C for 30 minutes, cooled in a desiccator and weighed.

Determination of phytochemical composition of ILV's

High Performance Liquid Chromatography (HPLC)

Extraction of Phenolic Compounds

Extraction of phenolic acids from the vegetables was carried out following the method of [19]. Briefly, 100 mg of milled samples were weighed into 20 ml amber EPA vials and extracted with 10 ml methanol: water (60:40). The mixtures were homogenized using a vortex for 30 seconds at 30 minute intervals. Samples were stored in the dark for 18 hours at room temperature. Samples were centrifuged at 3000 rpm/1400 g for 10 minutes. The supernatant was transferred to a volumetric flask, and the residue was re-extracted twice with 5 ml of methanol: water (60:40) mixture. The methanol extracts were pooled and methanol was evaporated using a rotary evaporator. The remaining residues were re-suspended in 2 ml methanol: water (50:50).

Determination of the Total Phenolic Content (TPC)

The total phenolic content of ILV's was determined using the Folin-Ciocalteu colorimetric method described by Singleton et al. [20]. Briefly, all extracts were diluted 1:20 with Milli-Q water in order to obtain readings that falls within the standard curve concentration range of 0.0–600.0 µg gallic acid/mL. The legume extracts were oxidized by the Folin-Ciocalteu reagent and then neutralized with sodium carbonate. The absorbance was measured at 760 nm, after 90 min at room temperature by using a ThermoScientific Genesys 20 (USA) spectrophotometer. The absorbance was compared with those of standards with known gallic acid concentrations. Total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight (DW) of sample. Data were expressed as mean ±SD of three replications.

The HPLC system employed was a 1100 HPLC (Agilent, Waldbronn, Germany) equipped with Chem-station Software, a model G1322A degasser and a model G131A diode array detection system to monitor at all wavelengths from 200 to 600 nm. For the column, Luna solution A, composed of 2% (v/v) acetic acid in water and solution B comprising of 0.5% acetic acid in water and acetonitrile (50:50, v/v) delivered at a flow rate of 1 ml/min as follows: initially 100% of solution A; for the next 15 min, 70% A; for another 30 min, 65% A; for another 20 min, 60% A; for another 5 min, 50% A; and finally 0% A for 25 min. The injection volume for the extract was 20 µL. Identification of each peak was confirmed using the retention time and absorbance spectrum of each pure compound. Pure standards used for the identification were, 3, 4-dihydroxy benzoic acid, rutin acid, vanillic acid, ferulic acid, p-coumaric acid, chlorogenic acid, nicotiflorin, 4-hydroxyl benzoic acid. The calibration curves were constructed with the specific wavelengths of standard chemicals: 280 nm for rutin, chlorogenic acid, 3,4-dihydroxybenzoic and 4-hydroxybenzoic Acids and 320nm for ferulic, chlorogenic, nicotiflorin, coumaric and vanillic acid. The samples were identified by retention time and absorbance spectrum. Results obtained for the sample extracts were expressed as mean ± SD for triplicates.

Statistical analysis

Statistical analysis were conducted using SPSS (Statistics for Social Science) version 18.0 for windows. Statistical significance was set at $p < 0.05$. All the results were presented as mean ± SD for at least triplicate for each sample.

RESULTS AND DISCUSSION

Proximate composition of ILV's

African Leafy Vegetables (ALVs) are considered as valuable sources of nutrients [21]. The proximate composition of ILV's is shown in Table 1. The findings of the moisture, ash, fiber, protein and fat content in ILV's are comparable to the previously published literature [7, 10]. The moisture content of the vegetables was high and ranged from 84.1 ± 0.05 (*Amaranthus spp.*) to 88.8 ± 0.10 (*Cleome gynandra*) indicating that the vegetable is susceptible to spoilage. This may be the reason why this vegetable is often sun-dried for few hours for storage purposes. Significant differences were observed in the ash content ($p < 0.05$) and ranged between 1.90 ± 0.06 % (*Amaranthus spp.*) and 3.0 ± 0.04 % (*Cleome gynandra*).

Table 1: Proximate Composition and Energy Value of ILV's (mean +SD, n=3)

Leafy vegetables	Moisture (% wet basis)	Ash (%)	Fat (g/100g)	Protein (g/100g)	Fibre (g/100g)
Amaranthus spp.	84.1 ± 0.05^a	1.90 ± 0.06^a	0.88 ± 0.10^a	5.60 ± 0.10^a	1.84 ± 0.50^a
Cleome gynandra	88.8 ± 0.10^a	3.0 ± 0.04^b	0.66 ± 0.03^b	6.42 ± 0.41^b	1.59 ± 0.40^b
Vigna unguiculata	85.4 ± 0.12^a	2.15 ± 0.50^a	0.82 ± 0.20^a	4.6 ± 0.70^c	1.2 ± 0.10^c

Values with different letters in each row are significantly different ($p < 0.05$)

All the leafy indigenous vegetables were found to be poor sources of protein, fat and fibre (Table 1). The crude protein content range from 4.6 ± 0.30 (*Vigna unguiculata*) to 6.42 ± 0.41 (*Cleome gynandra*) mg/100g. The fat content ranged from 0.42 ± 0.20 (*Vigna unguiculata*) to 0.88 ± 0.10 (*Amaranthus spp.*) mg/100g. These findings are in line with the findings of many authors which showed that leafy vegetables are poor sources of fat [7, 10, 22]. Diet low in fat is said to be beneficial to human beings, as excessive intake of fats can cause complications such as cancer [23] and obesity. Green leafy vegetables are very nutrient-dense and healthy and can therefore be recommended as part of weight reducing diets. However, Uusiku [10] asserts that people on high vegetable diets should be encouraged

to obtain their essential fatty acids from other sources such as oils from indigenous seeds. *Vigna unguiculata* had the highest fibre (4.22 ± 0.10 g/100g) compared to other two vegetables. The fibre content also compared favourably with the fibre contents of *Vigna unguiculata* and *Cleome gynandra* recorded by Uusiku et al. and van der Walt et al. respectively [10,12]. High levels of fibre in foods may aid digestion, and prevention of colon cancer and in the treatment of diseases such as obesity, diabetes and gastrointestinal disorders [24].

Phytochemical Composition of ILV's

Total Phenolic Content (TPC)

All the ILV's showed a significant amount of TPC (Table 2). Expressed as milligrams of gallic acid equivalent per gram of sample on DW basis, the total phenolics ranged from 10.4 ± 0.5 to 40.4 ± 0.11 mg/g.

Table 2: Total Phenolic Content of Legumes. Values Expressed as Milligrams of GA Equivalents/ g DW (mean \pm SD, n=3)

Leafy vegetables	Total phenolics (mg GAE/ g DW)
Amaranthus spp.	40.4 ± 0.11^a
Cleome gynandra	10.4 ± 0.5^b
Vigna unguiculata	19.1 ± 0.09^c

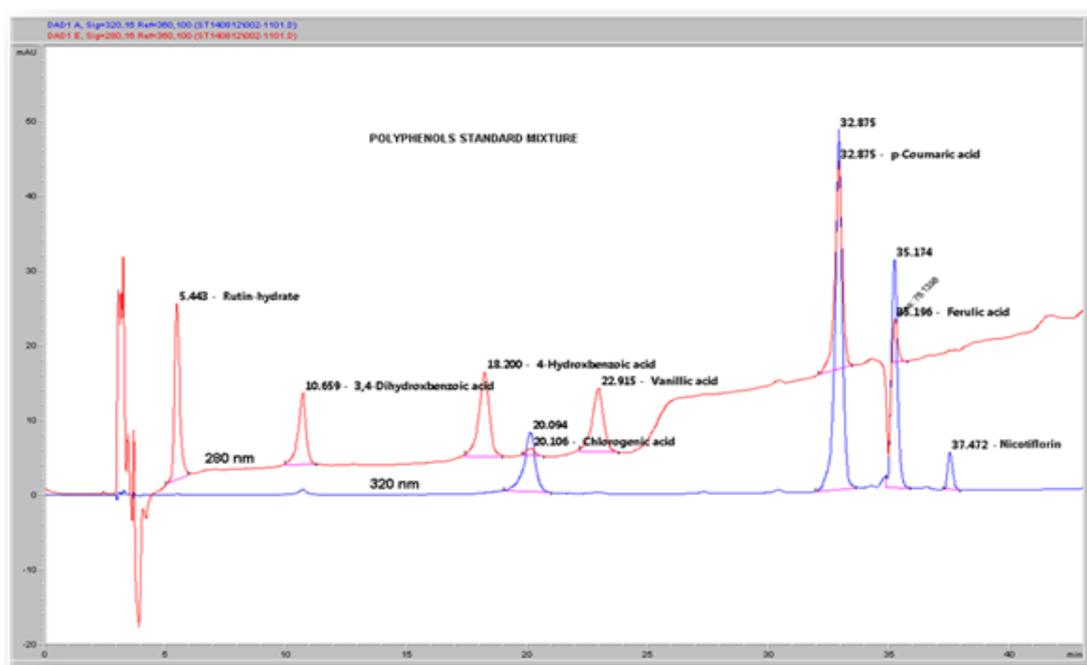
Values with different letters in each row are significantly different ($p < 0.05$)

Amaranthus spp. had the highest phenolic content (40.4 ± 0.11 mg/g) followed by *Vigna unguiculata* (19.1 ± 0.09 mg/g). The TPC value of *Amaranthus spp.* was comparable to those found in another study [22]. Van der Walt et al. [12] reported TPC value of 29.1 mg GAE/g, DW for 80% methanol extracts of cowpea leaves (*Vigna unguiculata*), which is higher than the 19.1 mg GAE/g, DW for the 75% acetone extracts of *Vigna unguiculata* reported in this study. Katerere et al. [25], also reported the concentration in *Vigna unguiculata* and *Amaranthus spp.* as 109.14 and 79.79 mg GAE/100g respectively after extraction with 70% methanol. *Cleome gynandra* had the lowest phenolic content (10.4 ± 0.5 mg/g), but the TPC value was similar to that previously reported by Chipurura [22]. However, Moyo et al. [26] reported TPC value of *Cleome gynandra* as 3.94 mg GAE/g which was lower than reported in this study. A wide variation was observed for phenolic contents of the vegetables under study, or even for the some vegetables reported by different authors. The differences might be due to factors such as environment [27], different groups of phenolic compounds in the plants, the methods of extraction and analysis [27,28] and assay types, maturity factors [29] and genetics [30]. Based on these results, HPLC analysis was employed to define qualitative content of phenolic acids of *Amaranthus spp.*, *Cleome gynandra*, and *Vigna unguiculata*.

HPLC analysis

The HPLC analysis of standards highlighted the quantifiable presence of 8 polyphenols classified as phenolic acids, hydroxybenzoic and hydroxycinnamates (Figure 1).

Figure 1: A standard mixture of polyphenols containing 100mg/L of Rutin, Vanillic, 3,4 Hydroxybenzoic acid, 4-Hydroxybenzoic, Chlorogenic, Nicotiflorin, *p*-Coumaric acids and ferulic acid.



The leafy vegetable polyphenols separated by HPLC was identified by comparing their retention time (RT) with those of pure standards while quantification was performed using the standard curve, based on a peak match against those of the standards. Calibration curves for the standards were obtained using a series of concentrations of these compounds at a range of 2.5 mg/L to 1000 mg/L. The calibration standards for the six standards were linear. The retention times, liner calibration curves and correlation coefficients (r²) for Polyphenols analysis are shown in Table 3.

Table 3: Retention Times, Linear Calibration Curves and Correlation Coefficients (r²) for Polyphenols Analysis. Area – Peak Area; Amount – Concentration (mg/100 g).

Polyphenols	Retention time (min)	Equation	Correlation coefficient
3,4-dihydroxybenzoic acid	10.659	Area = 27.344656 × amount - 159.903	0.99952
4-hydroxybenzoic acid	18.2	Area = 41.8506273 × amount - 138.29523	0.99964
Chlorogenic acid	20.094	Area = 35.0433044 × amount – 104.19823	0.99949
Vanillic acid	22.917	Area = 37.012224 × amount -166.51327	0.9997
P-Coumaric acid	32.875	Area = 99.4275202 × amount +119.26625	0.99956
Ferullic acid	35.174	Area = 66.72370 × amount -122.09955	0.99968

The calibration curves for the flavonoids rutin, nicotiflorin were not linear and therefore removed from the analysis. However, rutin and quercetin have been reported in *Amaranthus spp* [31]. In another study, the seeds of *Amaranthus hypochondriacus* were found to contain rutin, isoquercetin and nicotiflorin [32,33,34]. Rutin has been identified as having the potential to prevent and treat colorectal carcinogenesis [35].

Table 4 shows the polyphenols detected in the vegetable Samples. The phenolic compounds identified in this study are widely found in plants. Shahidi et al. [36] identified gallic acid, protochatechuic acid, ferulic acid and vanillic acid in tomatoes. For *Amaranthus spp.*, 3, 4 dihydroxybenzoic acid, chlorogenic acid and ferulic acid were found. Vanillic acid, chlorogenic acid and ferulic acid were found in *Cleome gynandra*. P-coumaric acid was also found but it was below the amount quantified. There were traces of p-coumaric acid in *Cleome gynandra*. P-coumaric has beneficial effect in protecting animals against doxorubicin (DOX)-induced cardiac oxidative damage [37]. *Vigna unguiculata* had the highest number of polyphenols namely 3, 4 dihydroxybenzoic acid, 4-hydroxybenzoic acid, ferulic acid and p-coumaric acid. Chlorogenic acid was the most abundant polyphenol.

Table 4: Composition of the phenolic compounds in ILV’s extracts expressed as milli grams per 100 gram of DW (mean ± SD, n=3) Revealed by HPLC.

Polyphenols*	Samples		
	<i>Amaranthus spp.</i>	<i>Cleome gynandra</i>	<i>Vigna Unguiculata</i>
3,4-dihydroxybenzoic acid	7.56 ± 0.61	ND	7.25 ± 0.11
4-hydroxybenzoic acid	ND	ND	3.85 ± 0.14
Vanillic	ND	1.13 ± 0.09	ND
Chlorogenic acid	12.34 ± 0.95	31.32 ± 4.42	ND
Ferulic acid	3.63 ± 0.09	2.38 ± 0.09	4.75 ± 0.25
P-coumaric acid	ND	ND	1.60 ± 0.03

*Phenolic acid content is expressed in mg/100 g, DW-dry weight basis

ND: Not detectable; Results are values of mean and ±standard deviation of triple determinations.

In another study, ferulic acid and p-coumaric acid were also detected from *Vigna unguiculata* and *Cleome gynandra* [38]. Ferulic acid, p-coumaric acid, vanillic acid, and 4-hydroxybenzoic acid were detected in seeds of *Amaranthus* genotypes [32] and similar results were reported in different species of *Amaranthus* using paper chromatography [39]. The latter results are in agreement with earlier published data [28]. Furthermore, research has shown that each type of vegetable has a wide range of variation between different phenolic compounds in their effectiveness as antioxidants [40]. The presence of the CH=CH-COOH group in the hydroxycinnamic acids (p-coumaric acid, ferulic acid) is considered to be key for the significantly higher antioxidative efficiency than the COOH in the hydroxybenzoic acids [41].

The fact that ferulic acid appeared in all of the vegetables analysed, emphasizes their potential role in the fight against cancer [42]. ILV’s thus appears to be a good source of antioxidant molecules such as polyphenols. However, there were a number of unidentified peaks in the extracts which were detected and they could be considered as potential polyphenols. It was recommended that the several unidentified peaks be further subjected to LC-MS analysis. Studies of polyphenol composition of ILV’s species similar to the ones used in this study and grown in Sub-Saharan Africa are limited, therefore it is difficult to make comparison of polyphenols levels found in this study with those in the literature from ILV’s grown elsewhere. It should also be mentioned that factors such as extraction methods, the type of solvent, the extraction time, as well as differences in genotypes, agronomic practices, environmental and climatic growth

conditions used, harvesting time, handling practices prior analysis and season of the year are also critical in making comparisons[43,44]. However, the phenolic compound detected in the ILV's is an indication that these vegetables have high antioxidant activity which can be useful for the prevention of cardiovascular and other chronic diseases.

CONCLUSIONS

The indigenous leafy vegetables studied here have appreciable nutrient and phenolic content. Inclusion of these vegetables in the daily diet will not only add on to the nutritional value of the diet but also serve as functional foods. It is critical to create awareness regarding diet related health benefits of these vegetables. Further studies to investigate the phenolic composition of these vegetables in the region are suggested in order to best characterize them. This is the first report on identification of phenolic acids in the extracts of four leafy vegetables grown in Botswana.

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